these data indicate that endocardium may not contribute significantly to cardiac CFU-Fs, the possibility remains that CFU-Fs of endocardial origin may be functionally different from epicardial CFU-Fs and require a different cell culture medium for optimal detection.

Understanding the developmental biology of the heart is instrumental in developing novel technologies for heart regeneration and cellular therapies. It is critical to identify the type and origin of cells capable of reconstituting a heart. To determine whether a cardiac mesenchymal cell compartment could be reconstituted by bone marrow cells after injury, Chong et al. (2011) performed CFU-F evaluation in the heart after transplantation of bone marrow cells following myocardial infarction, irradiation, and hematopoietic stem cell immobilization. While the authors found that bone marrow cells could reconstitute the marrow CFU-F compartment, they failed to detect their contribution to cardiac CFU-Fs. These findings support the view that bone marrow cells do not reconstitute cardiac cells after cardiac injury. In this context, the activation of cardiac resident progenitors could be considered as a reasonable approach to promote cardiac tissue regeneration and vessel growth. Recent work by Smart et al. (2011) found that expression of embryonic epicardial progenitor marker *Wt1* can be activated in the adult epicardium by stimulation with thymosin β4 prior to myocardial infarction. Subsequently, these cells migrate into the heart and contribute to smooth and cardiac muscles and interstitial fibroblasts. Further work will be required to determine the relationship between epicardial progenitors described by Smart and colleagues (Smart et al., 2011) and cCFU-Fs identified by Chong and colleagues (Chong et al., 2011). Do they represent similar or hierarchically related progenitors? Are there any stimuli that can activate cCFU-Fs and facilitate cardiac repair and vascularization after myocardial infarction? Since repair consists of a combination of regeneration and scar formation, it is important to determine the contribution of cCFU-Fs to postinfarct scar formation and whether improvement in restoration of cardiac function can be achieved by modulation of sclerogenic versus tissue-forming response of cCFU-Fs. Certainly, the findings presented by Chong et al. (2011) introduce several interesting and novel avenues of investigation, particularly in the context of cardiac regenerative therapies.

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Blood Cells Need Glia, Too: A New Role for the Nervous System in the Bone Marrow Niche

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Regulation of hematopoietic stem cell (HSC) dormancy by specific cell types in the hematopoietic niche remains poorly understood. Yamazaki et al. (2011) now report that nerve-associated nonmyelinating Schwann cells activate TGF-β to maintain dormant HSCs, suggesting that glia are novel players in the bone marrow niche.

Hematopoietic stem cells (HSCs) reside in specialized microenvironments, or niches, that ensure their localization, survival, controlled proliferation, and differentiation. Components of the bone marrow (BM) hematopoietic niche include mesenchymal stem cells (MSCs), which secrete HSC homing and maintenance factors; osteoblasts, which derive from MSCs and line the surface of the endosteum on the inside of the bone cavity; and sympathetic nerve fibers, which coordinate hematopoietic

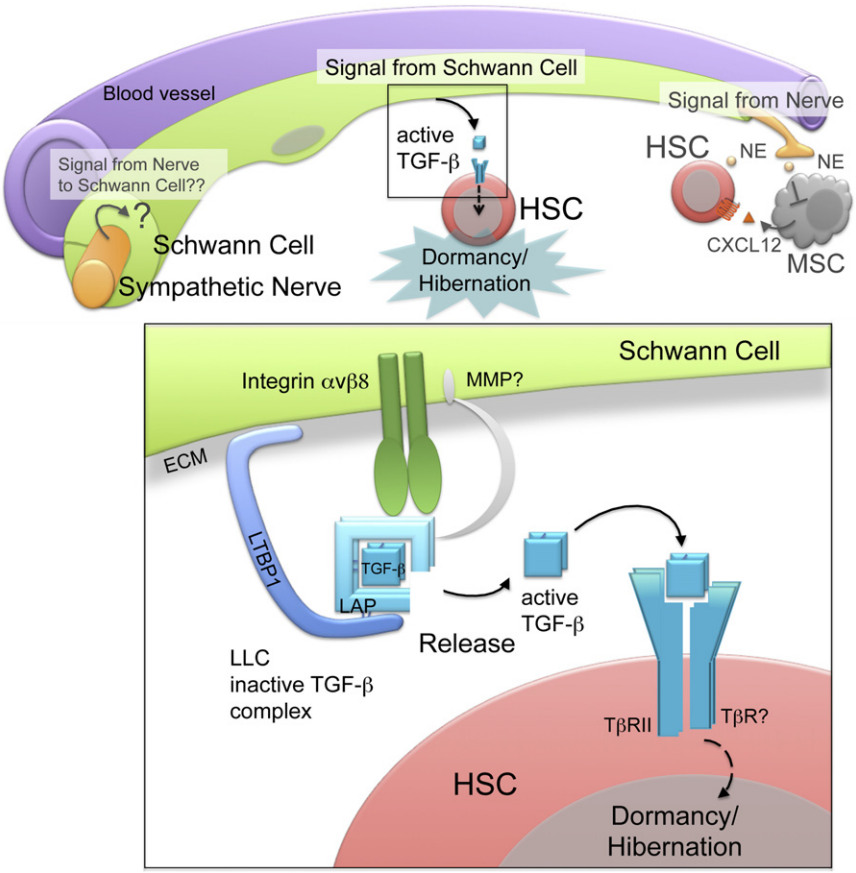

Figure 1. Model of Glia- and Nerve-Dependent Signals in the Bone Marrow Niche
Nonmyelinating Schwann cell (Schwann cell, green) ensheathing a sympathetic axon (nerve, orange), located in proximity of bone marrow vasculature (blood vessel, purple). Inactive TGF-β is part of the Large Latent Complex (LLC), in which LAP sequesters TGF-β, and LTBP-1 tethers LAP (and the associated TGF-β) to extracellular matrix (ECM). Schwann cells are thought to release active TGF-β via interactions with Integrin αvβ8 and a putative matrix metalloproteinase (MMP?). TGF-β binds to its cognate receptor on HSCs (composed of TβRII and an unknown type I receptor [TβR?]), triggering signaling pathways that ultimately result in HSC quiescence. "Signal from Nerve" summarizes previously described roles of neuron-produced neurotransmitters such as norepinephrine (NE) on MSCs, resulting in reduction of CXCL12 levels, which in turn affects HSCs, and on HSCs directly. Although currently unknown, signals from the sympathetic nerve may regulate Schwann cell activity ("Signal from Nerve to Schwann Cell??").

responses with systemic cues and central nervous system inputs (Ehninger and Trumpp, 2011; Katayama et al., 2006; Méndez-Ferrer et al., 2008, 2010). Consistent with differences in the proliferation of HSC populations, current models propose an "endosteal niche," which controls HSC quiescence or dormancy, and a "vascular niche" in the center of the bone cavity, which promotes HSC self-renewal (Ehninger and Trumpp, 2011). The precise cellular interactions and molecular mechanisms controlling the dormancy, or "hibernation," of HSCs remain elusive. In a recent study in *Cell*, Nakauchi and colleagues make the surprising finding that nonmyelinating Schwann cells, a type of glia wrapping around nerve fibers in the BM, constitute a novel functional component of the hematopoietic niche important for maintaining HSC dormancy (Yamazaki et al., 2011). Previously, the Nakauchi group reported that TGF-β signaling induces HSC quiescence by inhibiting raft microdomain clustering and reducing activation of src and signaling pathways that drive HSC proliferation (Yamazaki et al., 2009). Inactive TGF-β, in the form of a Large Latent Complex (LLC), is known to be produced by several cell types in the BM niche, including HSCs. However, which constituent of the hematopoietic niche would activate TGF-β and make it available to HSCs remained unclear (Yamazaki et al., 2009).

In their current paper, Nakauchi and colleagues reveal that nonmyelinating Schwann cells of the peripheral nervous system are the major source of active TGF-β in the BM. Schwann cells ensheath peripheral neurons of the autonomic, i.e., the sympathetic and parasympathetic, and the sensory nervous systems, by tightly wrapping around axons. Myelinating Schwann cells insulate larger axons but thin extensions such as the postganglionic sympathetic axons in the BM are ensheathed by nonmyelinating Schwann cells. Activation of TGF-β depends on its release from the LLC. In this complex, TGF-β is kept inactive by Latency Associated Protein (LAP), which derives from the TGF-β precursor and binds to Latent TGF-β Binding Protein 1 (LTBP-1), which tethers the complex to the extracellular matrix. Yamazaki et al. found that integrin β8, which is known to activate TGF-β from the LLC, was specifically expressed by nonmyelinating Schwann cells and colocalized with active TGF-β. Based on this, the authors propose a model in which integrin αvβ8 binds to LAP to enable activation of TGF-β, potentially involving proteolytic cleavage (Figure 1). The authors next took advantage of the fact that glia depend on trophic support from their associated neurons and performed unilateral transection of postganglionic sympathetic nerves in the lumbar trunk to reduce the number of active TGF-β producing Schwann cells in the BM. While the total number of BM cells was not affected by this procedure, relative numbers of HSCs in the BM were reduced and HSCs displayed reduced levels of TGF-β signaling as well as increased proliferation. This phenotype resembled that of HSCs under reduced TGF-β signaling conditions (*Tgfbr2*$^{+/-}$) and was reversed by prior infusion of active TGF-β in the denervated mice. BM denervation did not affect the repopulating capacity of the HSCs per se, nor did it trigger measurable egress of HSCs into the peripheral blood or spleen. These observations support the conclusion that active TGF-β produced by Schwann cells controls HSC dormancy in the BM but also raise some interesting questions. Although denervation did not significantly change the number of other niche cells, i.e., MSCs, osteoblasts, or endothelial cells, it seems possible that this procedure could have resulted in functional changes in these or other cell types of the hematopoietic microenvironment. Furthermore, lack of

sympathetic innervation in itself, independent from glia or TGF-β signaling, could also have contributed to the observed HSC defects.

In this context, it is noteworthy that several recent reports have described a functional role of the peripheral nervous system (PNS) in the hematopoietic niche (Katayama et al., 2006; Makhijani et al., 2011; Méndez-Ferrer et al., 2008, 2010; Spiegel et al., 2007). Studies by Frenette and colleagues demonstrated regulation of HSC trafficking and other behaviors by the sympathetic nervous system, both under physiological activation conditions such as circadian inputs from the central nervous system (Méndez-Ferrer et al., 2008) and under experimental challenges such as G-CSF stimulation (Katayama et al., 2006). In both instances, sympathetic adrenergic signals affect the expression of CXCL12 in MSCs or osteoblasts, resulting in reduced signaling through the corresponding receptor CXCR4 on HSCs and HSC egress from the BM (Katayama et al., 2006; Méndez-Ferrer et al., 2008, 2010). Sympathetic signals, such as the neurotransmitters dopamine and norepinephrine, can also trigger HSCs directly to regulate HSC proliferation, migration, and mobilization; their cognate receptors are expressed on HSCs, in particular under G-CSF-induced conditions (Spiegel et al., 2007).

Roles of the PNS in the hematopoietic niche may be widely conserved across phyla. Recently, genetics and live-imaging studies in the *Drosophila* larva showed that blood cells colonize hematopoietic "niches," i.e., segmentally repeated epidermal-muscular pockets jointly shared with cells of the PNS, and rely on the PNS for their localization and trophic survival (Makhijani et al., 2011). In vertebrates, the PNS is also a player in the microenvironments of secondary lymphoid organs and other, nonhematopoietic organ systems. Lymph nodes and spleen are highly innervated by sympathetic nerve fibers, which control the maturation and regulation of T cells and macrophages. This innervation provides a direct anatomical link allowing inputs from the central nervous system to regulate physiological and pathological immune responses (Straub, 2004). So far, in all these systems, attention has mostly been drawn to neuron-related functions. The findings by Yamazaki et al. challenge this existing view and encourage parallel studies on the role of glia relative to the contribution of neurons in PNS-dependent niches.

An outstanding question arising from the work by Nakauchi and colleagues is whether the activity of Schwann cells and the release of active TGF-β are regulated by neural activity of the nerve fibers they are ensheathing. In the PNS, sensory neurons of the dorsal root ganglia directly regulate their associated Schwann cells by ATP-based purinergic signaling and other neurotransmitters. This and similar neuron-glia communications do not require synaptic contacts and can take place at all neuron-glia interfaces, e.g., along nerve fibers or at the somata of neurons (Fields and Burnstock, 2006). By which mechanisms nervous inputs may control glial activity that affects the BM and other hematopoietic or nonhematopoietic niches will be an intriguing topic to explore in the future.

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